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CTCGAGGACAGTGACCTGGGAGTGAGTACAAGGTGAGGCCACCACTCAGGGT  
GCCAGCTCCAAGCGGGTCACAGGGACGAGGGCTGCAGGCCATCAGGAGGCCCT  
GCACACACATCTGGGACACGCGCCCCCGAGGGCAGTTCACCTCAGTGCAGCC  
TCATTCTCCTGCACAAAAGCGCCCCCATCCTTCTTCACAAGGCTTCGTGG  
AAGCAGAGGCAGTCGATGCCAGTACCCCTCCCTTCCCAGGCAACGGGACC  
CCAAGTTGCTGACTGGGACCACCAAGCCACGCATGCGTCAAGAGTGAGAGT  
CCGGGACCTAGGCAGGGCCCTGGGTTGGGCCTGAGAGAGAAGAGAACCTC  
CCCCAGCACTCGGTGTGCATCGTAGTGAAGGAGCCTCACCTGACCCCCGCT  
GTTGCTCAATCGACTTCCAAGAACAGAGAGAAAAGGAAACTTCAGGGCGG  
CCCCGGGCTCCTGGGGTTCCCACCCATTAGCTGAAAGCACTGAGGCA  
GAGCTCCCCCTACCCAGGCTCCACTGCCCGCACAGAAATAACAACCACGGT  
TACTGATCATCTGGGAGCTGTCCAGGAATTC

## FIG.\_1A

1 GCTGGGCTAA ACTGGGCTAG CCTGAGCTGG GCTGAACCTGG GCTGCTGGC  
51 TGGACTGGGT AAGCTGGCT GAGCTGGTT GGGTGGAAAT GGGCTGAGCT  
101 GAGCTAGGCT AAACCTGGTT TGGCTGGGCT GGGCTGGGCT GGG

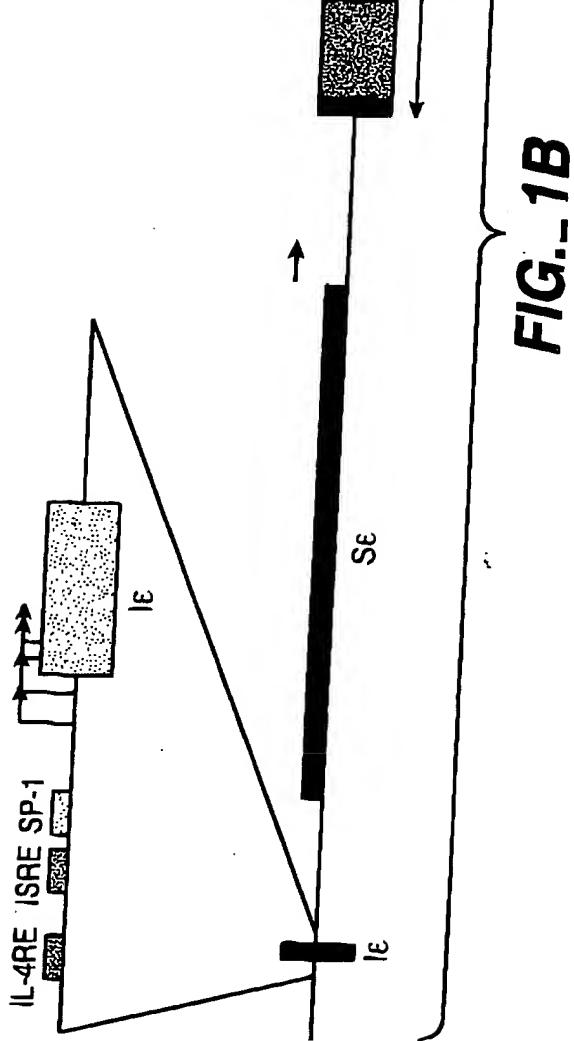
## FIG.\_2B

1 GGTTTGGCTG GGCTGGGCTG GGCTGGGCTG GGTCAGCTG AGCGGGTTGG  
51 GTTAGACTGG GTCAAACCTGG TTCAGC

## FIG.\_2C

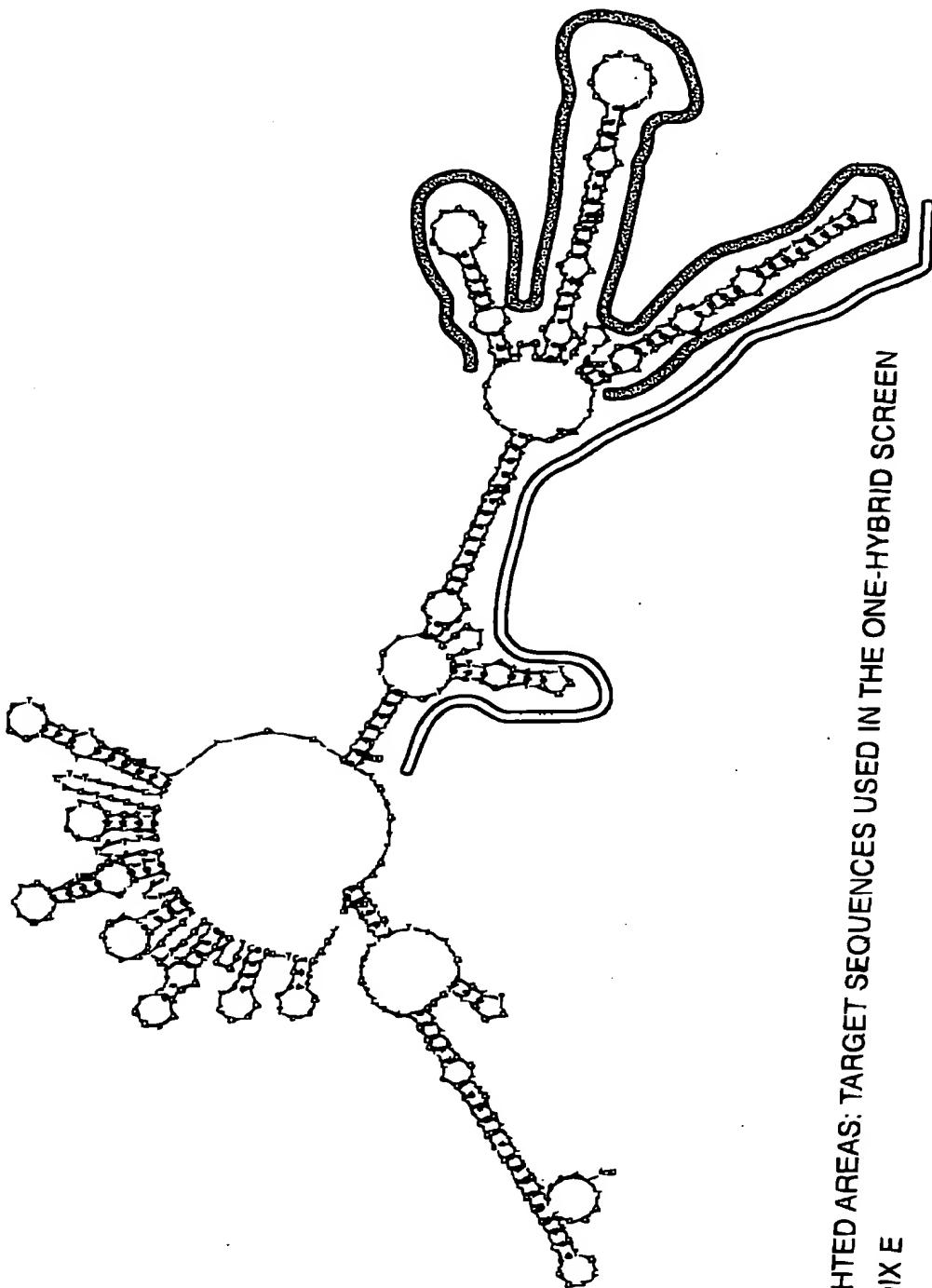
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GERMLINE & LOCUS



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LOW ENERGY DNA FOLDING OF THE S<sub>c</sub> REGION

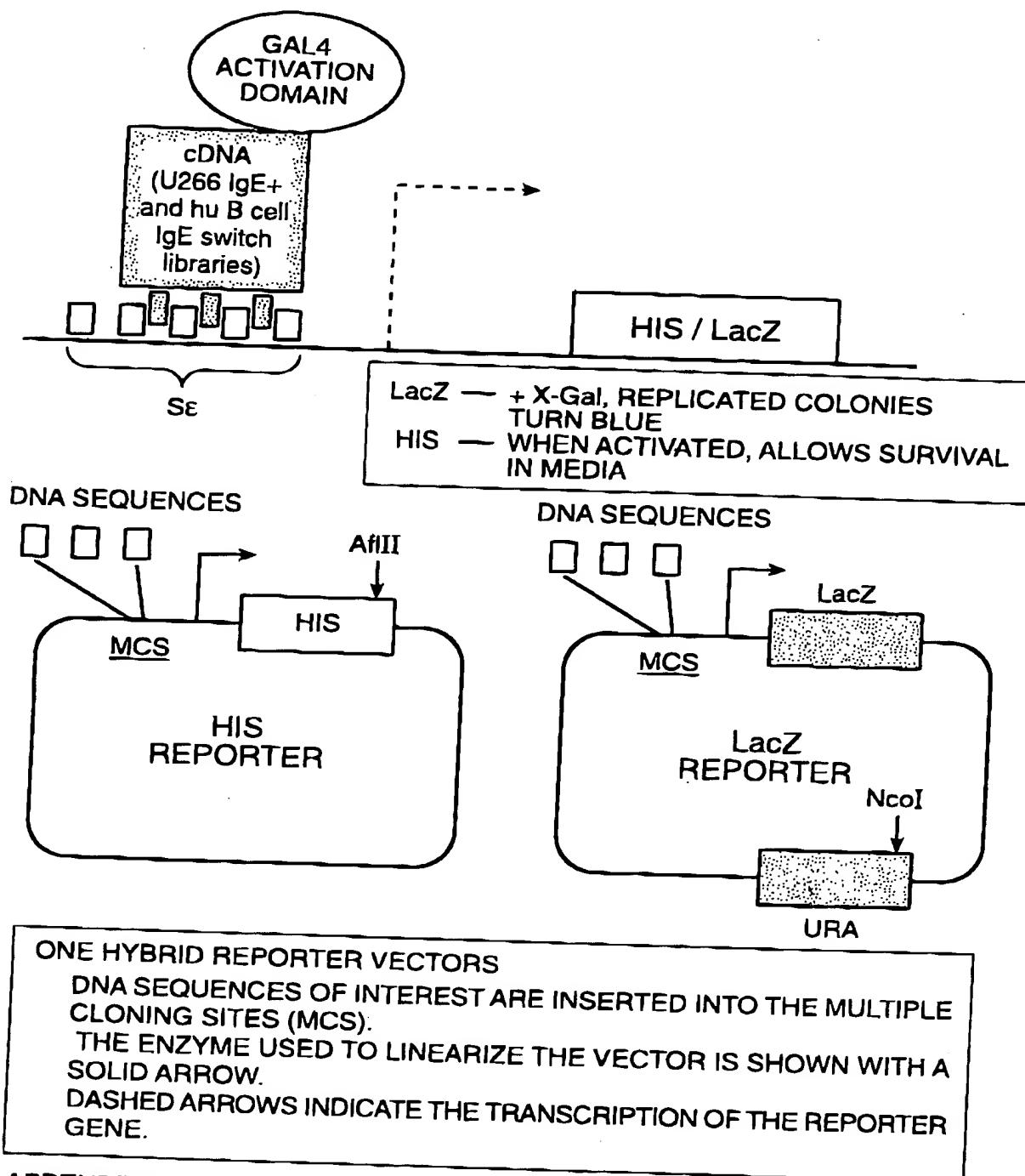


HIGHLIGHTED AREAS: TARGET SEQUENCES USED IN THE ONE-HYBRID SCREEN  
APPENDIX E

FIG. 2A

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## YEAST ONE-HYBRID SCREENING

**FIG.\_3**

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IL-4 INDUCTION OF GERMLINE  $\epsilon$  mRNA IN THE  
IgM + B CELL LINES: CA-46, MC-116 AND DND39

DND39 + IL-4

DND39 - IL-4

MC-116 + IL-4

MC-116 - IL-4

CA-46 + IL-4

CA-46 - IL-4

NEG. CONT.

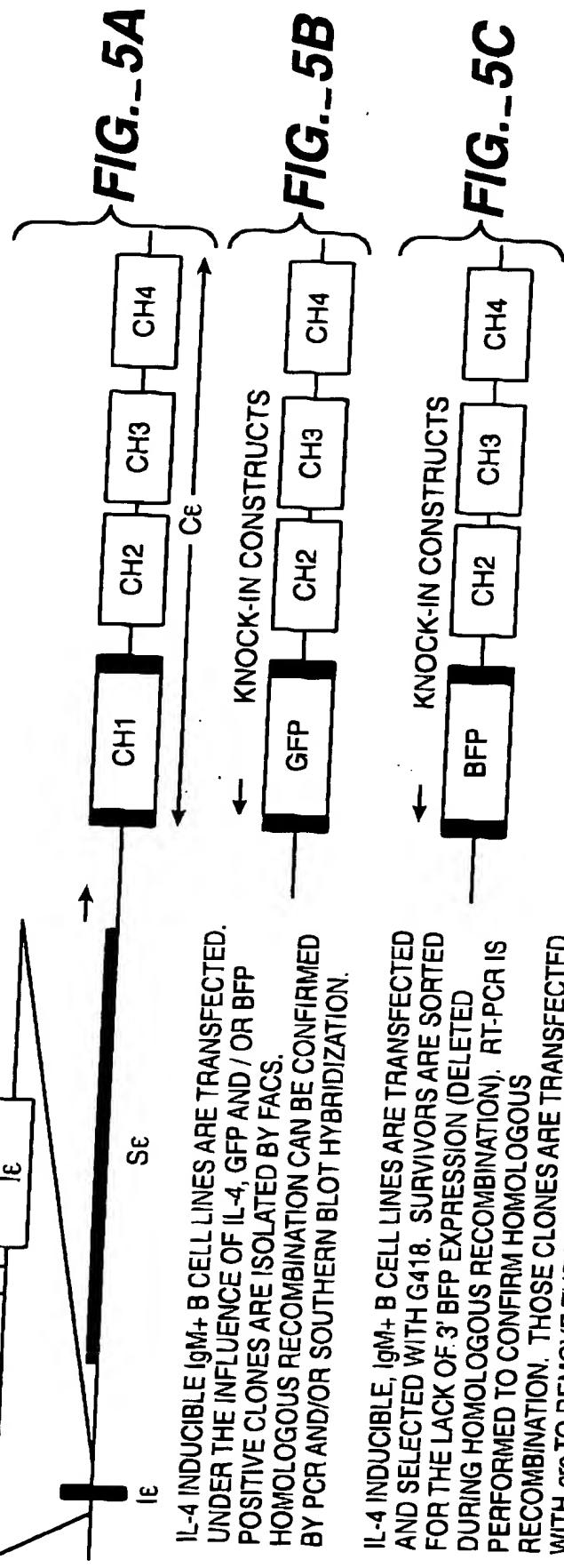


CELLS WERE INCUBATED FOR 48 HRS. IN 300 U / ml OF h-IL-4.  
RT-PCR WAS PERFORMED USING PRIMERS SPECIFIC FOR THE GERMLINE  
 $\epsilon$  EXON AND THE 5'-END OF THE C $\epsilon$  CH1 EXON (PREDICTED SIZE ~ 200 bp).

APPENDIX G

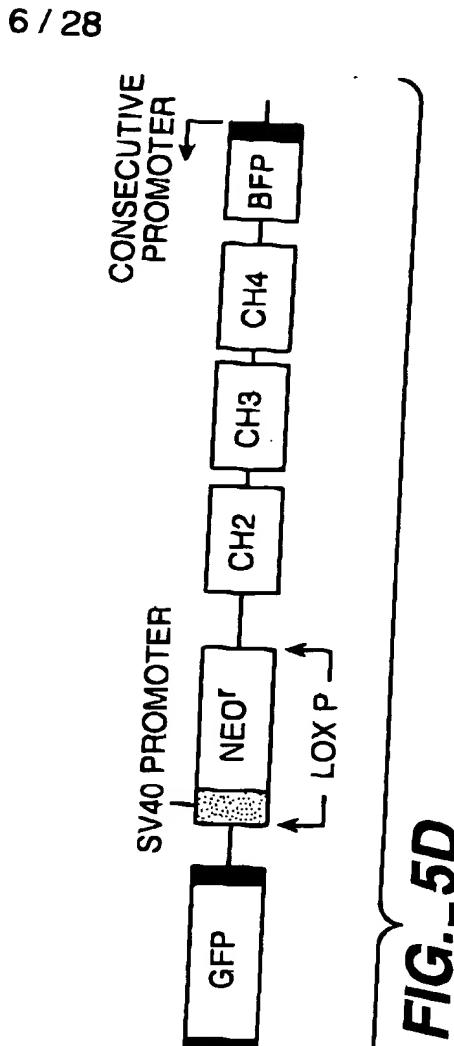
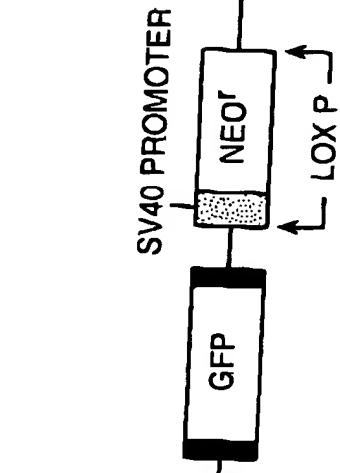
**FIG.\_4**

APPROACHES TO GENERATE GERMLINE & PROMOTER KNOCK-IN REPORTER CELL LINES



IL-4 RE, IL-4 RESPONSIVE ELEMENT  
IRE, INTERFERON RESPONSIVE ELEMENT  
SP-1, SP-1 BINDING SITE  
IE, NON-TRANSLATED EXON  
SE, SWITCH REGION OF ε  
GFP, GREEN FLUORESCENT PROTEIN  
BFP, BLUE FLUORESCENT PROTEIN  
CH 1, 2, 3, 4, CONSTANT REGION DOMAIN EXONS

APPENDIX A

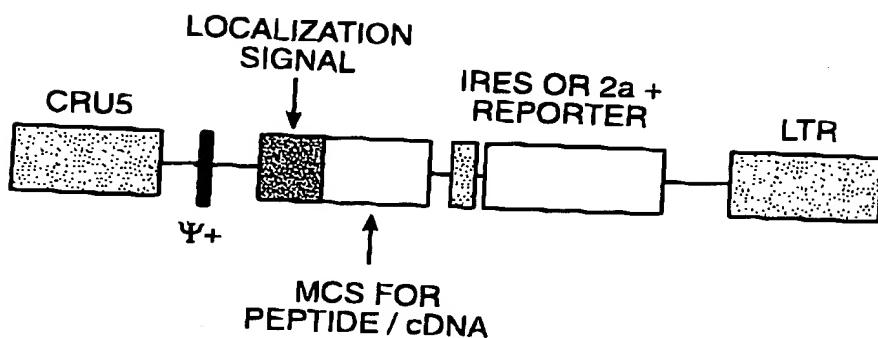


**FIG.-5D**

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### RIGEL BASE VECTOR



ALL COMPONENTS ARE UNIQUELY CASSETTED FOR FLEXIBILITY

CRU5, MODIFIED LTR  
LTR, LONG TERMINAL REPEAT  
Ψ+, PACKING SIGNAL  
LOCALIZATION SIGNAL: NUCLEAR, CELL MEMBRANE, GRANULAR  
MCS, MULTIPLE CLONING SITE  
IRES, INTERNAL RIBOSOME ENTRY SITE  
2a, SELF-CLEAVING PEPTIDE

APPENDIX I

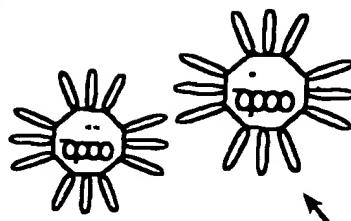
**FIG.\_6**

PROTOCOL FOR TRANSFECTION OF PHOENIX CELLS  
AND INFECTION OF NONADHERENT TARGET CELLS

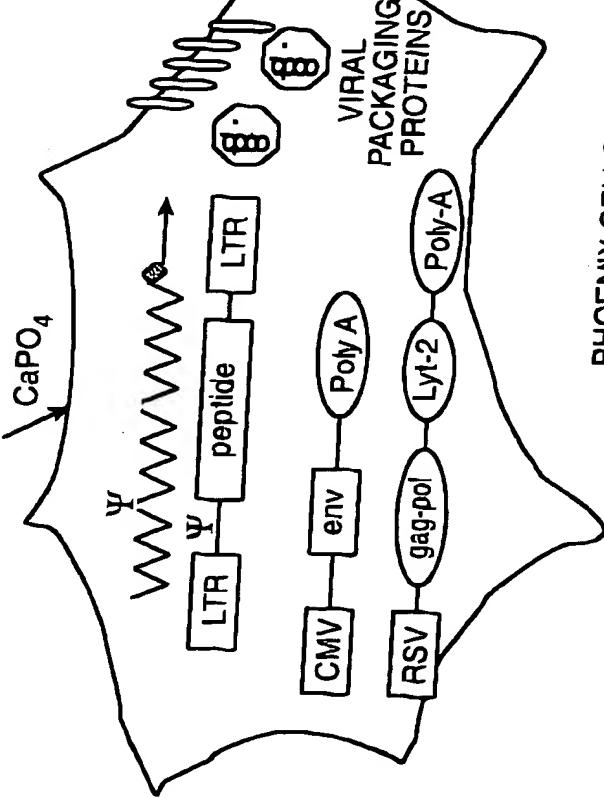
INFECTIOUS VIRAL LIBRARY



48 HOURS TO  
 $10^6 - 10^7$   
RETROVIRUSES / mL  
SUPERMANTANT



CaPO<sub>4</sub>



PHOENIX CELLS:  
LIBRARY PACKAGING CELL LINE

APPENDIX I

TARGET CELLS OF INTEREST

FIG. 7

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$\epsilon$  HEAVY CHAIN GFP / BFP KNOCK-IN CELL LINE  
U266  $\epsilon$  HEAVY CHAIN

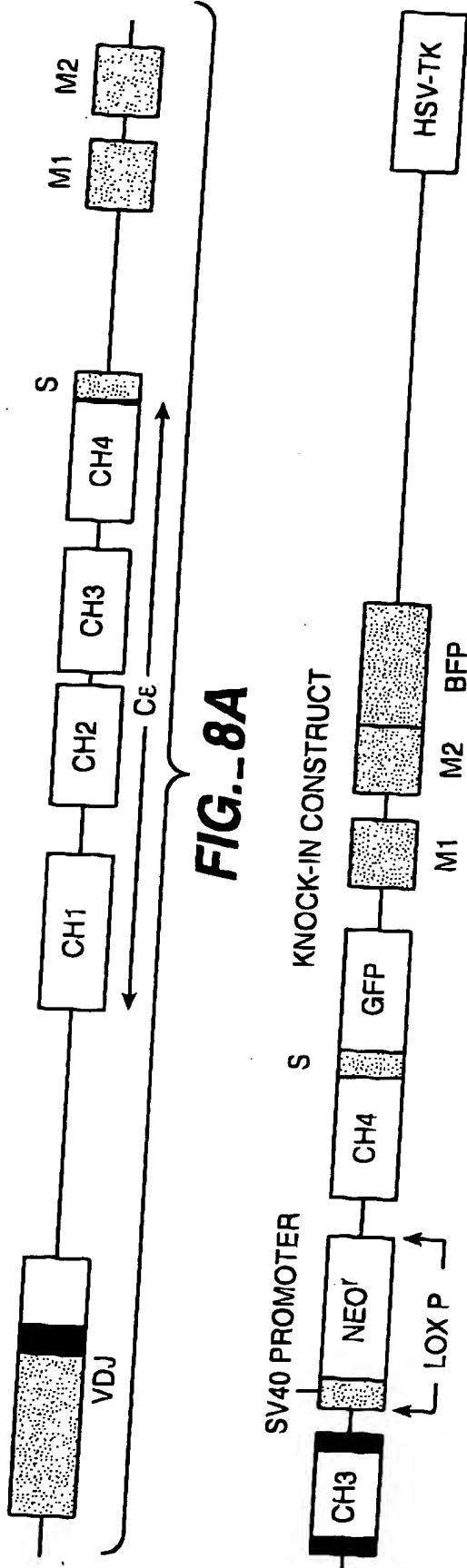


FIG.\_8A

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U266 CELLS ARE TRANSFECTED AND SELECTED WITH G418. SURVIVORS ARE TREATED WITH GANCICLOVIR (HSV-TK DELETED DURING HOMOLOGOUS RECOMBINATION). RT-PCR IS PERFORMED TO CONFIRM HOMOLOGOUS RECOMBINATION. THOSE CLONES ARE TRANSFECTED WITH Cre TO REMOVE THE SV40 NEOMYCIN RESISTANCE GENE.

APPENDIX D

S, SECRETORY EXON

GFP, GREEN FLUORESCENT PROTEIN

BFP, BLUE FLUORESCENT PROTEIN

Neo<sup>r</sup>, NEOMYCIN RESISTANCE GENE

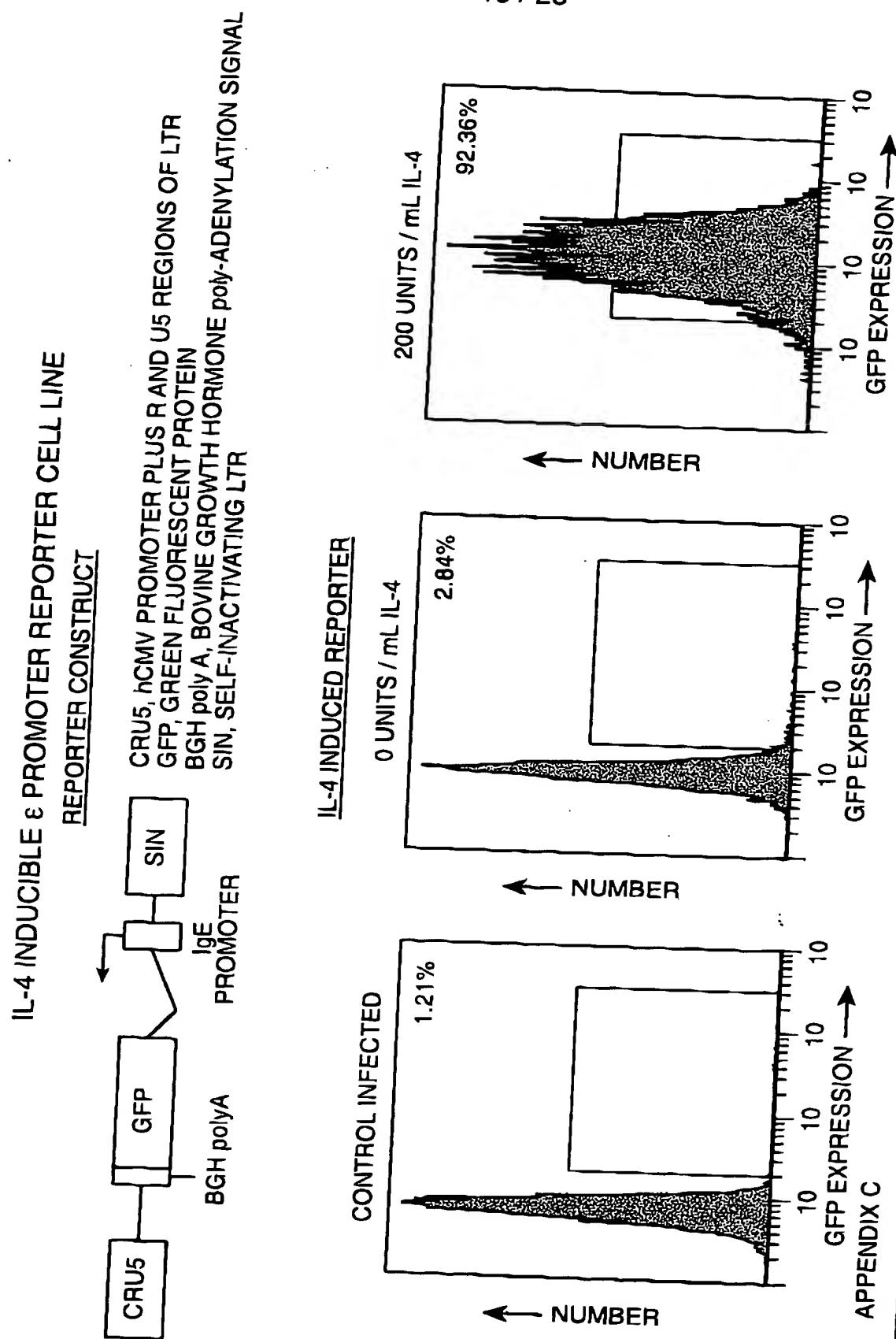
VDJ, V REGION EXON

CH 1, 2, 3, 4, CONSTANT REGION EXONS

M1, M2, MEMBRANE EXONS

HSV-TK, HERPES SIMPLEX VIRUS-THYMIDINE KINASE

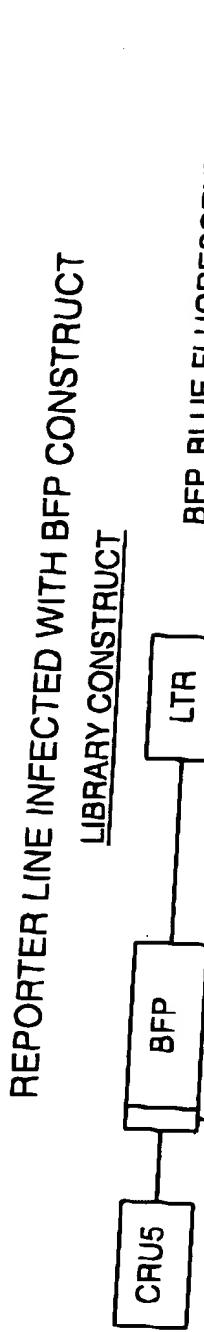
FIG.\_8B



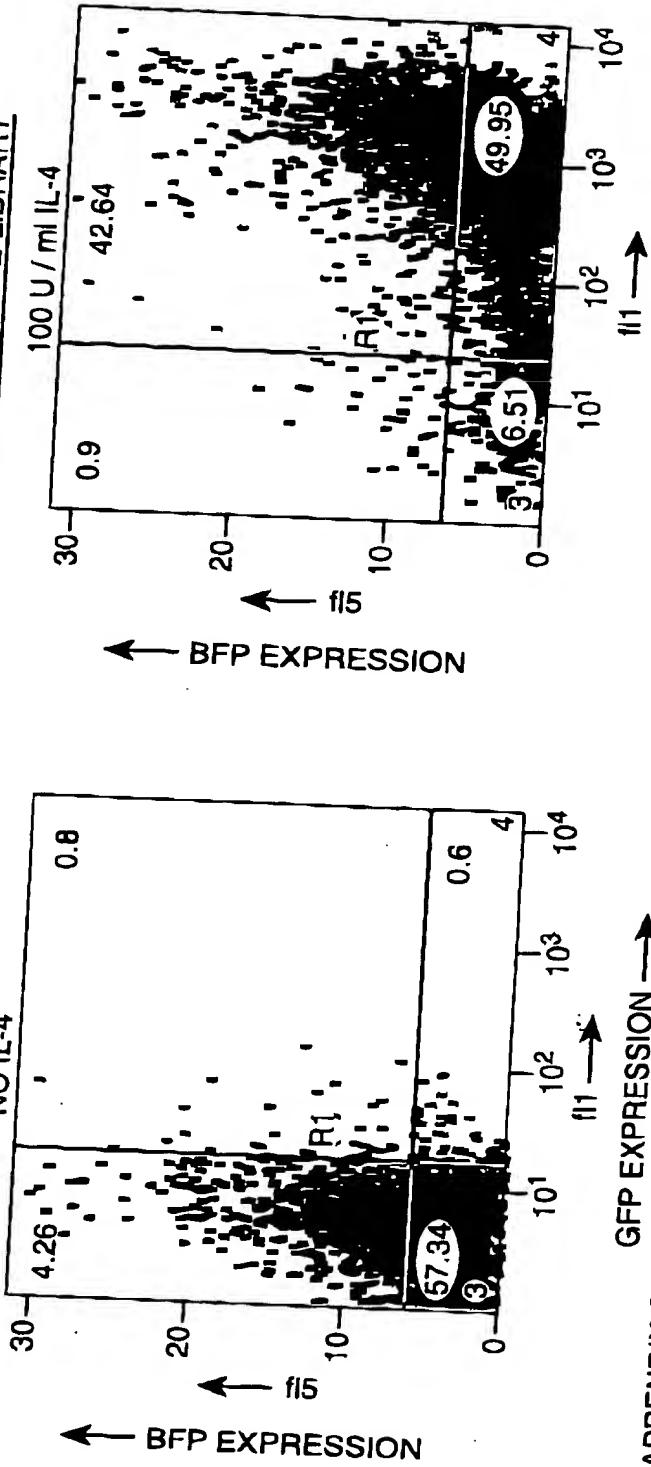
**FIG.- 9A**

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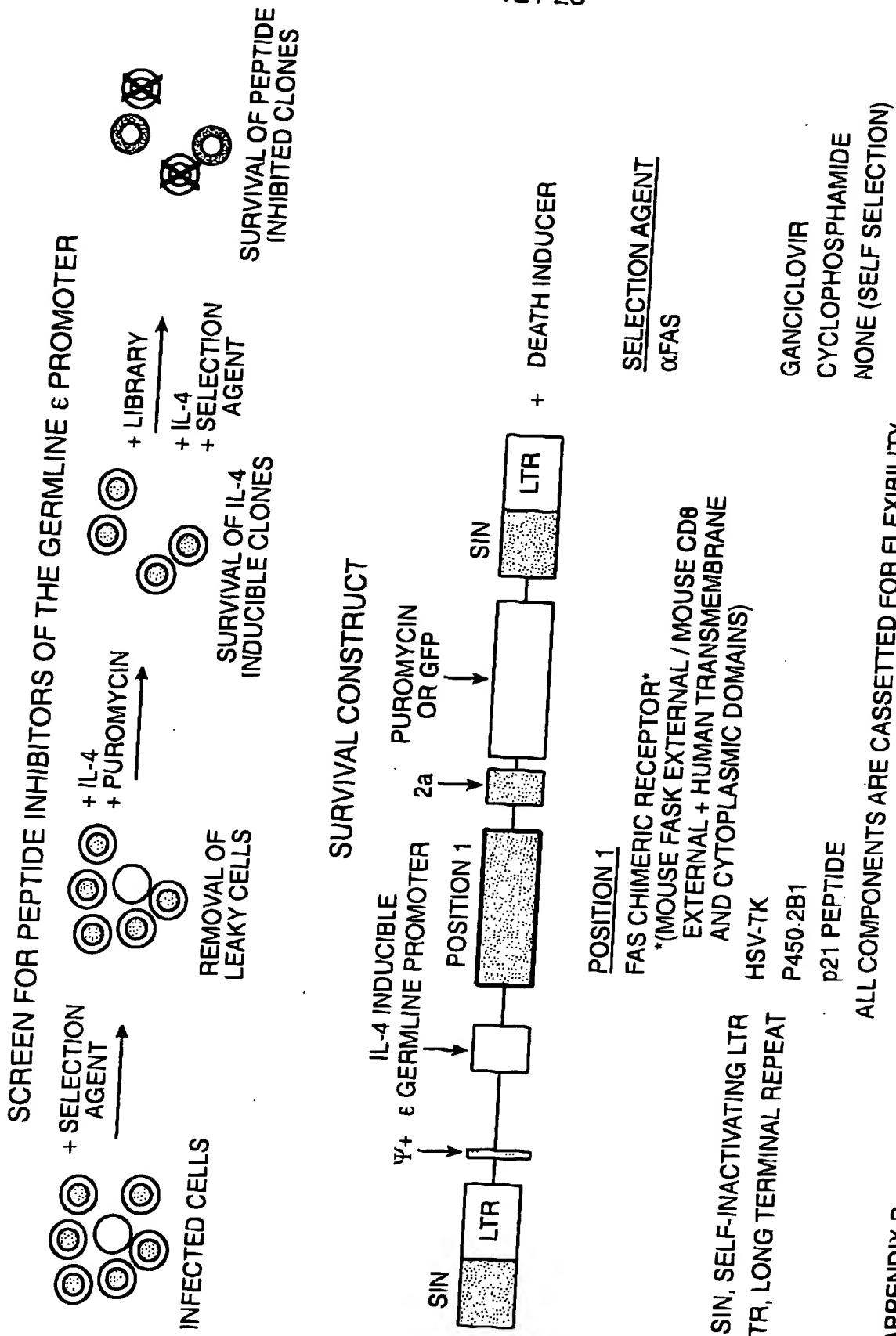
FACS PROFILE OF CELLS WITH BOTH REPORTER AND PEPTIDE LIBRARY



APPENDIX C

**FIG.\_9B**

GFP EXPRESSION →



**FIG.-10**

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- 1-845 CMV promoter/R/U5 5' LTR  
1322 GAG ATG-ATC mutation  
850-2100 extended Ψ region  
2146-2173 two BstX1 peptide cloning sites  
2205-2723 ECMV IRES (cloned as EcoR1/Msc1 fragment from pCITE-4a [Novagen])  
2746-3465 GFP coding region  
3522-4115 3' LTR  
4122-6210 pGEM backbone (pUC origin, ampR)

ATCACGAGGCCCTTCGTCTCAAGAACAGCTTGTCTTAGGAGTTCTAATACATCC  
CAAACCAAATATAAAAGCATTTGACTTGTCTATGCCCTAGTTATTAAATAGTAATCAA  
TTACGGGGTCATTAGTTCATAGCCCATAATGGAGTCCCGCTTACATAACTACGGTAA  
ATGGCCCGCCTGGCTGACCGCCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATG  
TTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTACGGT  
AAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTATTGACG  
TCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGACTTTC  
CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTATGCGGTTTGGC  
AGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCTCCACCCCA  
TTGACGTCAATGGGAGTTGTTGGCACCAAAATCAACGGACTTCCAAAATGTCGTA  
ACAACCTCCGCCATTGACGAAATGGCGGTAGGCATGTACGGTGGGAGGTCTATATAA  
GCAGAGCTCAATAAAAGAGCCCACAACCCCTCACTCGGGCGCAGTCCTCCGATTGACT  
GAGTCGCCGGTACCCGTATCCAATAACCCCTTTGCAGTTGCATCCGACTTGTGGT  
CTCGCTGTTCTGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAAGGGGGGCTTT  
CATTTGGGGGCTCGTCGGGATCGGAGACCCCTGCCAGGGACCACCGACCCACCG  
GGAGGTAAGCTGCCAGCAACTTATCTGTGTCTGTCCGATTGTCTAGTGTCTATGACTGA  
TTTATGCGCCTGCGTCGGTACTAGTTAGCTAACTAGCTCTGTATCTGGGGACCCGTGG  
TGGAACTGACGAGTTCGGAACACCCGGCGCAACCCCTGGGAGACGTCCCAGGGACTTCGG  
GGGCGTTTTGCGCCGACCTGAGTCAAAATCCGATCGTTGGACTCTTGGTCT  
CACCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAACAGTTCC  
CGCCTCCGTCTGAATTTCGTTCTGGTTGGGACCGAAGCCGCCGCGGTCTGTCT  
GCTGCAGCATCGTTCTGTGTCTGTCTGACTGTGTTCTGTATTGTCTGAAAATA  
TCGGCCCGGGCCAGACTGTTACCAACTCCCTTAAGTTGACCTTAGGTCACTGGAAAGATG  
TCGAGCGGATCGCTACAACCAAGTCGGTAGATGTCAAGAACAGACGTTGGGTTACCTTCT  
GCTCTGCAGAACGCCAACCTTAACGTCGGATGGCCGGAGACGGCACCTTAACCGAG  
ACCTCATACCCAGGTTAAGATCAAGGTCTTACCTGGCCGATGGACACCCAGACC  
AGGTCCCCCTACATCGTGAACCTGGGAAGCCTGGCTTTGACCCCCCTCCCTGGGTCAAGC  
CCTTGACACCCCTAACGCTCCGCCCTCTCCCTCCATCCGCCCTCTCTCCCCCTTG  
AACCTCCTCGTTGACCCGCCCTCGATCTCCCTTATCCAGCCCTCACTCCTCTCTAG  
GCGCCCCATATGCCATATGAGATCTTATATGGGGCACCCCCCTTGTAACACTTCC  
CTGACCTGACATGACAAGAGTTACTAACAGCCCTCTCTCCAGCTACCAAGAACAACTGG  
TCTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGAGCCTACCAAGAACAACTGG  
ACCGACCGGTGGTACCTCACCCCTACCGAGTCGGGACACAGTGTGGGTCCGCCGACACC  
AGACTAACCTAGAACCTCGCTGGAAAGGACCTACACAGTCCTGCTGACCAACCCCA  
CCGCCCTCAAAGTAGACGGCATCGCGCTGGATACACGCCGCCACGTGAAGGCTGCCGA  
CCCCGGGGTGGACCATCCTCTAGACTGCGGATCTGAGGGATCCACCAACCATGGACCC  
CCATTAAATTGGAATTCTGCAGCCGGGGATCCACTAGTTCTAGAGCGAATTAAATTCC

FIG.\_ 11A-1

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GGTTATTTCCACCATATTGCCGTCTTGGCAATGTGAGGGCCCGAAACCTGGCCCTG  
TCTCTTGACGAGCATTCTAGGGCTTCCCCTCGCCAAAGGAATGCAAGGTCTGT  
TGAATGTCGTGAAGGAAGCAGTCCCTGGAAGCTCTTGAAGACAAACAACGTCTGTAG  
CGACCCCTTGCAGGCAGCGGAACCCCCCACCTGGCAGAGGTGCCTCTGGCCAAAAGC  
CACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGA  
TAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGCTGAAGGATG  
CCCAGAAGGTACCCATTGTATGGATCTGATCTGGGCCTCGGTGCACATGCTTACAT  
GTGTTAGTCGAGGTTAAAAACGTCTAGGCCCGAACACGGGACGTGGTTTACAT  
TTGAAAAAACACGATGATAATATGGGGATCCACCCTGGTGCACCATGGTGAGCAAGGGCG  
AGGAGCTGTTCACCGGGGTGGTGCCCATTCTGGTCAGCTGGACGGCACGTAACGGCC  
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCCTGA  
AGTTCATCTGCAACCACGGCAAGCTGCCGTGCCCTGGCCACCCCTGTGACCAACCTGA  
CCTACGGCGTGCAGTCTCAGCCGTACCCGACCACATGAAGCAGCACGACTTCTCA  
AGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTCAAGGACGACGGCA  
ACTACAAGACCCGGCGCCGAGGTGAAGTTGAGGGGACACCCCTGGTAACCGCATCGAGC  
TGAAGGGCATCGACTTCAAGGAGGACGGCAACATCTGGGGACAAGCTGGAGTACAAC  
ACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAAC  
TCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTGCCGACCAACTACCAGCAGA  
ACACCCCCATCGGCGACGGCCCGTGTGCTGCCGACAACCAACTACCTGAGCACCCAGT  
CCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGTGAGTTGTA  
CCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCGGGCGTCGACGA  
TAAAAATAAAAGATTTATTTAGTCTCCAGAAAAAGGGGGAAATGAAAGACCCACCTGTA  
GGTTTGGCAAGCTAGCTTAAGTAACGCCATTTGCAAGGCATGGAAAAATACATAACTGA  
GAATAGAGAAGTTCAGATCAAGGTCAAGGAACAGATGGAACAGCTGAATATGGGCCAAACA  
GGATATCTGTGGTAAGCAGTTCTGCCCGGCTCAGGGCAAGAACAGATGGAACAGCTG  
AATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTGCCCGGCTCAGGGCAAGAA  
CAGATGGTCCCAGATGCGGTCCAGCCCTCAGCAGTTCTAGAGAACCATCAGATTT  
CAGGGTCCCCAAGGACCTGAAATGACCCCTGTGCTTATTGAACTAACCAATCAGTTG  
CTTCTCGCTTCTGTTCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCC  
TCACTGGGGGCCAGTCTCCGATTGACTGAGTCGCCGGTACCGTGTATCCAATAA  
ACCCCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTTCTGGGAGGGTCTCCTCTGA  
GTGATTGACTACCGTCAGGGGGTCTTCATTCCGACTTGTGGTCTCGCTGCCCTGG  
GAGGGTCTCTTGAGTGAATGACTACCGTCAGGGGGTCTTCACATGCAGCATGTAT  
AAAAATAATTGGTTTTCTTAAGTATTACATTAATGGCCATAGTTGCATTAAT  
GAATCGGCCAACGCGCGGGAGAGGCAGGGTTGCGTATTGGCCTTCCGCTTCCTCGCT  
CACTGACTCGCTCGCTCGGTGTTGGCTGCGAGCGGTATCAGCTCACTCAAAGGC  
GGTAATACGGTTATCCACAGAATCAGGGATAACGCAGGAAAGAACATGTGAGCAAAAGG  
CCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGGTTTTCCATAGGCTCCG  
CCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCAAACCGACAGG  
ACTATAAAAGATACCAAGGCGTTCCCGATACCTGTCGCCCTTCTCCCTCGGGAGCGTGGCG  
CCTGCCGCTTACCGGATACCTGTCGCCCTTCTCCCTCGGGAGCGTGGCGTTCTCA  
TAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGCTGT  
GCACGAACCCCCCGTTCAGCCGACCGCTGCGCTTATCCGTAACATCGTCTTGAGTC  
CAACCCGGTAAGACACGACTTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAG  
AGCGAGGTATGTAGGCGGTGCTACAGAGTTCTGAAGTGGTGGCCTAACTACGGCTACAC

**FIG.\_ 11A-2**

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TAGAAGGACAGTATTGGTATCTCGCTCTGCTGAAGCCAGTTACCTCGGAAAAAGAGT  
TGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTGTTGCAA  
GCAGCAGATTACGCGCAGAAAAAGGATCTCAAGAAGATCCTTGATCTTCTACGGG  
GTCTGACGCTCAGTGGAACGAAAACACGTTAAGGGATTTGGTCATGAGATTATCAA  
AAGGATCTCACCTAGATCCTTTAAATTAAAATGAAGTTGCGCAAATCAATCTAAAG  
TATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTC  
AGCGATCTGTCTATTCGTTCATCCATAGTTGCCTGACTCCCCGTGTTAGATAACTAC  
GATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTC  
ACCGGCTCCAGATTATCAGCAATAAACCGCCAGCCGAAGGGCCAGCGCAGAAGTGG  
TCCTGCAACTTATCCGCCATCCAGTCTATTAAATTGTTGCCGGGAAGCTAGAGTAAG  
TAGTTGCCAGTTAATAGTTGCGAACGTTGTTGCCATTGCTACAGGCATCGTGGTGT  
ACGCTCGTCTTGGTATGGCTTCATTGCTCCGGTCCAACGATCAAGGCAGTTAC  
ATGATCCCCATGTTGCAAAAAGCGGTTAGCTCCTCGGTCTCCGATCGTTGTCAG  
AAGTAAGTTGGCCGCAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTAC  
TGTCTGCCATCCGTAAGATGCTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTG  
AGAATAGTGTATGCCAGACCGAGTTGCTCTGCCCGCGTCAACACGGGATAATACCGC  
GCCACATAGCAGAACTTAAAGTGCCTCATCATTGAAAACGTTCTCGGGCGAAAAC  
CTCAAGGATCTACCGCTGTTGAGATCCAGTTGATGTAACCACTCGTGCACCCAACTG  
ATCTCAGCATCTTACTTCAACAGCGTTCTGGGTGAGCAAAACAGGAAGGCAAA  
TGCCGCAAAAAGGGATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTT  
TCAATATTATTGAAGCATTATCAGGGTTATTGTCATGAGCGGATACATATTGAATG  
TATTAGAAAATAACAAATAGGGGTTCCGCGCACATTG

FIG.- 11A-3

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1-845 CMV promoter/R/U5 5' LTR  
1322 GAG ATG-ATC mutation  
850-2100 extended ψ region  
2151-2865 GFP coding region  
2866-2894 GGGSGGG linker  
2895-2952 FMDV 2a cleavage sequence  
2953-3004 BstX1/BstX1/Hind3/Hpa1/Sal1/Not1 polylinker  
3052-3645 3' LTR  
3652-5715 pGEM backbone (pUC origin, ampR)

ATCACGAGGCCCTTCGTCTCAAGAACAGCTTAGGAGTTCTAATACATC  
CCAAACTCAAATATAAAGCATTTGACTTGTCTATGCCCTAGTTATAATAGTAATC  
AATTACGGGGCATTAGTCATAGCCCCATATGGAGTTCCCGCGTTACATAACTTACGG  
TAAATGGCCCGCCTGGCTGACCGCCCCAACGACCCCCGCCATTGACGTCAATAATGACG  
TATGTTCCCAGTAAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATT  
ACGGTAAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTTA  
TTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTATGG  
GACTTCCCTACTTGGCAGTACATCTACGTATTAGTCATCGTATTACCATGGTGTGCG  
GTTTGGCAGTACATCAATGGCGTGGATAGCGGTTGACTCACGGGGATTCCAAGTC  
TCCACCCATTGACGTCAATGGGAGTTGTTGGCACCAAAATCAACGGACTTCCA  
AAATGTCGTAACAACCTCCGCCATTGACGCAAATGGCGGTAGGCATGTACGGTGGGA  
GGTCTATATAAGCAGAGCTCAATAAAAGAGCCCACAACCCCTACTCGGGCGCCAGTC  
CTCCGATTGACTGAGTCGCCGGGTACCGTGTATCCAATAACCCCTCTGAGTTGCA  
TCCGACTTGGGTCTGCTGTTCTGGGAGGGTCTCTGAGTGTGATTGACTACCGT  
CAGCGGGGGCTTCTGGGAGGTAAAGCTGCCAGCAAATTATCTGTGTCTGCGATTGTC  
ACCGACCCACCACCGGGAGGTAAAGCTGCCAGCAAATTATCTGTGTCTGCGATTGTC  
TAGTGTCTATGACTGATTTATGCGCCTGCGTCTGGTACTAGTTAGCTAACTAGCTCTGT  
ATCTGGCGGACCGTGGAACTGACGGAGTTCGGAACACCCGGCGCAACCCCTGGAG  
ACGTCCCAGGGACTTCGGGGGCCGTTTGTGGCCGACCTGAGTCAAATCCGAT  
CGTTTGGACTCTTGGTGCAACCCCTTAGAGGGAGGATATGTGGTTCTGGTAGGAGA  
CGAGAACCTAAAACAGTTCCGCCCTCGTCTGAATTTGCTTGGTTGGGACCGAA  
GCCGCGCCGCGGTCTGTCTGCTGCAGCATCGTCTGTGTTCTGTCTGACTGTG  
TTCTGTATTGTCTGAAAATACGGCCCGGGCAGACTGTTACCAACTCCCTTAAGTT  
GACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTACAACCAGTCGGTAGATGTCA  
AGAAGAGACGTTGGGTTACCTCTGCTCTGAGAATGCCAACCTTAACGTCGGATGG  
CCGCGAGACGGCACCTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTC  
ACCTGGCCCGCATGGACACCCAGACCAGGTCCCTACATCGTACGCTGGGAAGCCTTGG  
CTTTGACCCCTCCCTGGGTCAAGCCCTTGTACACCTTAAGCCTCCGCTCCTCTT  
CCTCCATCCGCCCGTCTCTCCCTTGAAACCTCCTCGTACCCGCGCTCGATCCTC  
CCTTATCCAGCCCTCACTCCTCTAGGCGCCCCATATGCCATATGAGATCTTAT  
ATGGGGCACCCCGCCCTTGAAACTTCCCTGACCTGACATGACAAGAGTTACTAAC  
AGCCCTCTCTCCAAGCTCACTTACAGGCTCTACTTAGTCCAGCACGAAAGTCTGGAG  
ACCTCTGGCGGGAGCCTACCAAGAACAACTGGACCGACCGGTGGTACCTCACCCCTTAC  
GAGTCGGCGACACAGTGTGGGTCCGCCGACACCAGACTAAGAACCTAGAACCTCGCTGG  
AAAGGACCTTACACAGTCCTGCTGACCACCCCAACGCCCTCAAAGTAGACGGCATCGC  
AGCTTGGATACACGCCGCCACGTGAAGGCTGCCGACCCCCGGGGTGGACCATCCTTA  
GACTGCCGGATCTGAGGGATCCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGG

**FIG. 11B-1**

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GTGGTGCCCCATCTGGTCAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTG  
CGGCAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTCATCTGCACCA  
CGGGCAAGCTGCCCGTGCCT GCCCACCCCTCGTACCGACCACCTGACCTACGGCGTGCAG  
TGCTTCAGCCGCTACCCGACCACATGAAGCAGCACGACTTCTCAAGTCCGCCATGCC  
CGAAGGCTACGTCCAGGAGCGCACCACATCTTCTCAAGGACGACGGCAACTACAAGACCC  
GCGCCGAGGGTGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATC  
GACTTCAGGGAGGACGGCAACATCCTGGGGACAAGCTGGAGTACAACATAAACAGCCA  
CAACGTCTATATCATGGCGACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATCC  
GCCACAAACATCGAGGGACGGCAGCGTGCAGCTCGCCGACCACTACCGAGCAGAACACCCCC  
ATCGGCAGGGCCCCGTGCTGCTGCCGACAACCACTACCTGAGCACCCAGTCCGCC  
GAGCAAAGACCCCACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGAACCGCC  
CCGGGATCACTCTCGGCATGGACGAGCTGTACAAGGAATTGGAGACGTCAGTCCAACCC  
GGTCAGCTGTTGAATTGACCTTCTAAACTTGGGAGACGTCAGTCCAACCC  
GCCACACCACCATGGAAGCTCCATTAAATTGGTAAACGTCAGCGGGCCGCTCGAC  
GATAAAATAAAAGATTATTAGTCCTCAGAAAAAGGGGGAAATGAAAGACCCCACCT  
GTAGGTTGGCAAGCTAGCTTAAGTAACGCCATTGCAAGGCATGGAAAAATAACATAA  
CTGAGAATAGAGAAGTTCAGATCAAGGTAGGAACAGATGGAAACAGCTGAATATGGG  
AAACAGGATATCTGGGTAAGCAGTTCTGCCCGGCTCAGGGCCAAGAACAGATGGAA  
CAGCTGAATATGGGCAAACAGGATATCTGTGGTAAGCAGTTCTAGAGAACCC  
CCAAGAACAGATGGTCCCCAGATGGGTCCAGCCCTCAGCAGTTCTAGAGAACCC  
GATGTTCCAGGGTCCCCAAGGACCTGAAATGACCTGTGCTTCTAGAGAACCC  
ATCAGTTCGCTTCTCGCTTCTGTTCGCGCTCTGCTCCCCGAGCTCAATAAAAGAGC  
CCACAACCCCTCACTCGGGCGCCAGTCCTCGATTGACTGAGTCGCCGGGTACCGT  
GTATCCAATAACCCCTTGCAGTTGCATCCGACTTGTGGTCTCGCTGTCTTGGAG  
GGTCTCTCTGAGTGATTGACTACCGTCAGGGGGCTTTCTAGTACATTAAATGGC  
CTCGCTGCTTGGGAGGGTCTCTGAGTGATTGACTACCGTCAGGGGGCTTTCA  
CATGCAGCATGTATCAAATTAAATTGGTTTTTCTAAGTATTACATTAAATGGC  
CATAGTTGCATTAATGAATCGGCCAACCGCGGGGAGAGGCAGGTTGCATGGG  
CTTCCGCTTCCCGCTCACTGACTCGCTCGCTCGGTGCTGCCGAGCGGTA  
TCAGCTCACTCAAAGCGGTAATACGGTTATCCACAGAACGAGGATAACGCAGGAAA  
GAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAGGCCGTTGCTGG  
CGTTTCCATAGGCTCCGCCCGTACAGGACTATAAGATACCAAGGCGTTCCCG  
AGGTGGCGAAACCCGACAGGACTATAAGATACCAAGGCGTTCCCG  
CGTGCCTCTCTGTTCCGACCCCTGCCGTTACCGATACTGTCGCC  
CGGGAAAGCGTGGCGTTCTCATAGCTACGCTGTAGGTATCTCAGTTCGGTG  
GTTCGCTCCAAGCTGGGTGTGACGAACCCCCGTTAGCCGACCGCTGCC  
ATCCGGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTATGCC  
CAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGT  
AAGTGGTGGCCTAACTACGGCTACACTAGAACGAGTATTGGTATCTGC  
GAAGCCAGTTACCTCGGAAAAAGAGTTGGTAGCTTGTACCGG  
CTGGTAGCGGTGGTTTTGTTGCAAGCAGCAGATTACCGCAGAAAAAGG  
CAAGAAGATCCTTGTACGGGTCTGACGCTAGTGGAAACGAAACTC  
TTAAGGGATTGGTACGAGATTATCAAAGGATCTTACCTAGATC  
AAAAATGAAGTTGCGAAATCAATCTAAAGTATATGAGTAAACT  
TACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTG  
AGTTGCCTGACTCCCCGTGAGATAACTACGATAACGGAGGG  
CCAGTGCTGCAATGATAACCGGAGACCCACGCTCACCG  
GCCAGATTATCAGCAATA

FIG.\_ 11B-2

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AACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGCCTGCAACTTATCCGCCTCCAT  
CCAGTCTATTAAATTGTTGCCGGGAAGCTAGAGTAAGTAGTCGCCAGTTAATAGTTGC  
GCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGGCTCGTGTGGTATGGCT  
TCATTCACTCCGGTCCCACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAA  
AAAAGCGGTTAGCTCCTTCGGTCCGATCGTTGTACAGAAGTAAGTGGCCGCAGTGT  
TATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGA  
TGCTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCCGGCG  
ACCGAGTTGCTCTTGCCCCGGGTCAACACGGGATAATACCGGCCACATAGCAGAACTT  
TAAAAGTGCATCATGGAAAACGTTCTCGGGGGCGAAAACCTCTCAAGGATCTTACCG  
CTGTTGAGATCCAGTTCGATGTAACCAACTCGTGCACCCAACGTATCTCAGCATT  
TACTTCAACCAGCGTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGAAAAAGG  
GAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTCCTTTCAATATTATTGA  
AGCATTATCAGGGTATTGTCATGACATTAACCTATAAAATAGGCGT

**FIG.\_ 11B-3**

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1-845 CMV promoter/R/U5 5' LTR  
 1322 GAG ATG-ATC mutation  
 850-2100 extended ψ region  
 2146-2173 two BstX1 peptide cloning sites  
 2173-2214 EcoR1/Apa1/Hpa1/Not1 polylinker  
 2262-2855 3' LTR  
 2855-4901 pGEM backbone (pUC origin, ampR)

ATCACGAGGCCCTTCGTCTCAAGAACAGCTTGCTTAGGAGTTCTAATACATC  
 CCAAACCTAAATATAAAGCATTTGACTTGTCTATGCCCTAGTTATAATAGTAATC  
 AATTACGGGGTCATTAGTCATGCCATATGGAGTTCCCGCTTACATAACTACGGT  
 AAATGGCCCGCCTGGCTGACCGCCAACGACCCCCGCCATTGACGTCAATAATGACGT  
 ATGTTCCCAGTAAACGCCAATAGGGACTTCCATTGACGTCAATGGGTGGAGTATTAA  
 CGGTAAACTGCCCACTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCTAT  
 TGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTATGGG  
 ACTTCCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCCG  
 TTTGGCAGTACATCAATGGGCGTGGATAGCGGTTGACTCACGGGATTCCAAGTCT  
 CCACCCATTGACGTCAATGGGAGTTGGCACCAAAATCAACGGACTTCCAA  
 AATGTCGTAAACAATCCGCCATTGACGCAAATGGCGGTAGGCATGTACGGTGGAG  
 GTCTATATAAGCAGAGCTCAATAAGAGGCCACAACCCCTACTCGGGCGCCAGTCC  
 TCCGATTGACTGAGTCGCCGGTACCGGTATCCAATAACCCCTTGCAGTGCAT  
 CCGACTTGTGGTCTCGCTGTTCTGGGAGGGTCTCTGAGTGATTGACTACCCGTC  
 AGCGGGGGTCTTCATTGGGGCTGTCCGGGATCGGGAGACCCCTGCCAGGGACCA  
 CCGACCCACCACCGGGAGGTAAGCTGCCAGCAACTTATGTGTCTGTCCGATTGTCT  
 AGTGTCTATGACTGATTATGCGCCTGCGTGGTACTAGTTAGCTAACTAGCTCTGTA  
 TCTGGCGGACCCGTGGTGGAACTGACGAGTTGGAACACCCGGCGAACCCCTGGGAGA  
 CGTCCCAGGGACTTCGGGGCGTTTGTGGCCGACCTGAGTCAAAATCCCGATC  
 GTTTGGACTCTTGGTGCACCCCTTAGAGGAGGGATATGTGGTTCTGGTAGGAGAC  
 GAGAACCTAAACAGTCCCGCCTCGTGAATTGGCTTCGGTTGGGACCGAAG  
 CCGCGCCGCGCGTCTGTCTGCGCATCGTCACTGTTCTGTCTGACTGTGT  
 TTCTGTATTGTCTGAAAATATCGGCCGGGCCAGACTGTTACCAACTCCCTTAAGTTG  
 ACCTTAGGTCACTGGAAAGATGTCGAGCGGATCGCTCACACCAGTCGGTAGATGCAA  
 GAAGAGACGTTGGTTACCTCTGCTCTGCAGAATGGCCAACCTTAACGTCGGATGGC  
 CGCGAGACGGCACCTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTCA  
 CCTGGCCCGCATGGACACCCAGACCAGGGTCCCTACATCGTACCTGGGAAGCCTTGGC  
 TTTGACCCCTCCCTGGTCAAGCCCTTGACACCCCTAACGCTCCGCTCCTCTTC  
 CTCCATCCGCCCGTCTCTCCCTTGACACCTCTCGTGGACCCCGCTCGATCCTCC  
 CTTTATCCAGCCCTCACCTCTCTAGGCGCCCCATATGCCATATGAGATCTTATA  
 TGGGGCACCCCGCCCTGTAAACTCCCTGACCTGACATGACAAGAGTTACTAAC  
 GCCCCTCTCTCCAAGCTCACTTACAGGCTCTACTTAGTCCAGCACGAAGTCTGGAGA  
 CCTCTGGCGGAGCCTACCAAGAACAACTGGACCGACCGGGTGGTACCTCACCCCTAACG  
 AGTCGGCGACACAGTGGGGTCCGCCGACACCAGACTAACGAAACCTAGAACCTCGCTGGA  
 AAGGACCTTACACAGTCCTGCTGACCAACCCACCGCCCTAAAGTAGACGGCATCGCA  
 GCTTGGATACACGCCGCCACGTGAAGGGTGCCTGACCCGGGGTGGACCATCCTCTAG  
 ACTGCCGGATCTCGAGGGATCCACCAACATGGACCCCTTAAATGGAATTGGGGCC  
 CAAGCTTGTAAACGTCGACGCCGCCGTCACGATAAAATAAAAGATTATTTAG  
 TCTCCAGAAAAAGGGGGAAATGAAAGACCCACCTGAGGTTGGCAAGCTAGCTTAAG  
 TAACGCCATTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTCAAGATCA

FIG.- 11C-1

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AGGTCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAG  
TTCCTGCCCGGCTAGGGCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGA  
TATCTGTGGTAAGCAGTTCTGCCCGGCTAGGGCAAGAACAGATGGTCCCCAGATG  
CGGTCCAGCCCTCAGCAGTTCTAGAGAACCATCAGATGTTCCAGGGTGCCCCAAGGA  
CCTGAAATGACCCCTGTGCCTATTGAACTAACCAATCAGTCGTTCTCGCTCTGTT  
CGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCACAACCCCTACTCGGGGGGCC  
AGTCCCTCCGATTGACTGAGTCGCCGGGTACCGTGATCCAATAACCCCTTGAGT  
TGCATCCGACTTGTGGTCTCGCTGTTCTGGGAGGGTCTCTGAGTGATTGACTAC  
CCGTCAAGCGGGGGTCTTCATTCCGACTTGTGGTCTCGCTGCCCTGGGAGGGTCTCCT  
CTGAGTGATTGACTACCCGTCAAGCGGGGTCTCACATGCAGCATGTATCAAATTAAAT  
TTGGTTTTTTCTTAAGTATTACATTAAATGGCCATAGTTGCATTAATGAATCGGCC  
AACGCGCGGGGAGAGGGCGGTTGCGTATTGGCGCTCTCCGCTTCGCTCACTGACT  
CGCTGCGCTCGGTGTTCGGCTGCGGAGCGGTATCAGCTCACTCAAAGGCAGTAATA  
CGGTTATCCACAGAACAGGGATAACGCAGAAAAGAACATGTGAGCAAAGGCCAGCA  
AAAGGCCAGGAACCGTAAAAGGCCGCGTTGCGTGGCGTTTCCATAGGCTCCGCCCC  
CTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCAAACCCGACAGGACTA  
TAAAGATAACCAGCGTTCCCGTGGAGCTCCCTCGTGCGCTCTCCGTGTTCCGACCC  
GCCGCTTACCGGATAACCTGTCGCGCTTCTCCCTCGGAAGCGTGGCGCTTCTCATA  
GCTCACGCTGTAGGTATCTCAGTTGCGTAGGTCGTCGCTCCAAGCTGGTGTG  
CACGAACCCCCCGTTCAAGCCGACCGCTGCCCTATCCGTAACATATCGTCTTGAGTC  
CAACCCGGTAAGACACGACTTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCA  
GAGCGAGGTATGTAGGCAGGTACAGAGTTCTGAAGTGGTGGCCTAACTACGGCTAC  
ACTAGAAGGACAGTATTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTCGGAAAG  
AGTTGGTAGCTCTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTGT  
GCAAGCAGCAGATTACCGCAGAAAAAGGATCTCAAGAACGATCCTTGATCTTCT  
ACGGGGTCTGACGCTCAGTGGAACGAAAACACGTTAAGGGATTGGTCAATGAGATT  
ATCAAAAAGGATCTCACCTAGATCCTTTAAATTAAAAATGAAGTTGGCAGAACCAA  
TCTAAAGTATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCA  
CCTATCTCAGCGATCTGTCTATTGTTCATCCATAGTTGCGCTGACTCCCCGTCGTGA  
GATAACTACGATAACGGGAGGGCTTACCATCTGCCCGAGTGCTGCAATGATACCGCGAG  
ACCCACGCTCACCGGCTCCAGATTATCAGCAATAAACAGCCAGCCAGCGGAAGGGCGAG  
CGCAGAAGTGGTCTGCAACTTATCCGCTCCATCCAGTCTATTAAATTGTTGCCGGGA  
AGCTAGAGTAAGTAGTCGCCAGTTAATAGTTGCGCAACGTTGTTGCCATTGCTACAG  
GCATCGTGGTGTACGCCGCTCGTTGGTATGGCTTCAATTGCTCCGGTCCAAACGA  
TCAAGGCAGTTACATGATCCCCATGTTGCAAAAAGCGGTTAGCTCCTTCGGTCC  
TCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTATCACTCATGGTTATGGCAGCAC  
TGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTCTGTGACTGGTGAGTAC  
TCAACCAAGTCATTCTGAGAATAGTGTATGCCGAGCGAGTTGCTCTGCCCGCGTC  
AACACGGGATAATACCGGCCACATAGCAGAACCTTAAAAGTGTCTCATATTGGAAAAC  
GTTCTGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGTCTGATGTA  
CCCACCGTGCACCCAACTGATCTCAGCATTTACTTCACCAAGCGTTCTGGGT  
AGCAAAAACAGGAAGGCAAAATGCCGAAAAAGGGAATAAGGGGACACGGAAATGTT  
GAATACTCATACTCTTCTTTCAATATTATTGAAGCATTATCAGGGTTATTGTC  
ATGACATTAACCTATAAAATAGGGGT

FIG.-11C-2

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## (1) C125cPaa Survival connector

C12scFas: epsilon-cFas(CD95)-Ires-Rygro-BGH PolyA put into C12B vector backwards so that no leaky transcription happens through the cmv promoter.

TGGATGCAATTCCCTCATTTTAACTTCCCTGGCTGCCCCACCCAGAATAGATCTAGACAA

FIG. 12A

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CAGGGTGGCAGTTTGCTTTCGCGGTTGACTGGTATTCTGGGT

TCTTGTCGCCATGTACTCCGTTGGT

AACCCGGCTCCTAGCTTAAACTCTGGAGATGCTATTAGTACCTTGAGTATGAACTGTTTCAG

**FIG. 12B**

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**FIG. 12C**

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FIG. - 12D

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## (2) Ahhh: Survival construct

TGGGATGCAATTCCCTCATTTTATTAGGAAGGACAGTGGAGTTGGCACCTTCAGGGTCAAAGGAAGGCACGGGGAGGG  
GAAAACAAACAGATGGCTGGCAACTTCAAGGAAAGGCACTTCAAGGAAAGGCACTTCAAGGAAAGGCACTTCAAGGAAAGG

**FIG.-13A**

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CCCTTTTCGGGAGCTAAATAAATCTTATTATCgtatagatccggcatctacttttccttgcctcg  
ggcattttttcggttccactatcggggatccggacagtccggatccggatccggatccggatccggatccgg  
gaaattggcgtaaccaaggctccgttggatagggtggatcgaaatggggatctggatccggatccggatccgg  
cgaccctcgatggcgttggatccggatccggatccggatccggatccggatccggatccggatccggatccgg  
attgttggagccggatccggatccggatccggatccggatccggatccggatccggatccggatccggatccgg  
cctgg  
aaatcacggccatccgg  
ggcgatgg  
ccctccctacatcgaaaggccatccgg  
cgatcgaaacttccgg  
cacgtccccgg  
atcagatcccatacaatgg  
tttccacaacttccaaactcacaacgtggggactggggactggggatccggggatccggggatccgggg  
ccggaggggccctgtggccagggtgg  
ttccAGGGGAACTGGCTTCTTCACGACATTCAACAGAACCTTGATTCGGGAGGGGAAGAACCCctagacctaga  
ccgggtttggatttcatctgtgaatgttt  
tctgtggaaatgggttt  
ccaaatatacgaaaggcagggtggacttt  
tgacaccatcttttcgaaaaaaaggcttt  
tcbaatcgataaaatt  
tt  
ccaaaccgggtttccgggtcccttccatcgaggccacgggggggggggggggggggggggggggggggg  
tgcaatcgatgg  
gtacatccggatccggatccggatccggatccggatccggatccggatccggatccggatccggatccgg  
acttt  
ggggcccaacacttccatcgatgg  
atggccatggggatccatggggatccatggggatccatggggatccatggggatccatggggatccatgg  
aacggggccatggggatccatggggatccatggggatccatggggatccatggggatccatggggatccatgg  
GAGTGGACACCTGTGGAGAGAAAGGCAAGGGCAAGGGGAAAGGGGAAAGGGGAAAGGGGAAAGGGGAAAGGG  
CTGTCCTCGACAGGCCAGTTCTATTGGTCTCCTTAACCTGTACTTACCTGCTTGGCCAGTGCCTCACG  
FIG.-13B

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FIG.-13D